THE VETERINARY JOURNAL

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Vol. 102 September, 1946 No. 9

EDITORIAL

TUBERCULIN

WE publish in this number of the *Veterinary Journal* a comprehensive article by Dr. H. H. Green on tuberculin: in the article are described some of the methods used in the past in preparing this product as well as those in use now in America and in England. In order that the results of tuberculin-testing of cattle can be assessed to the best advantage and that repeated tests in the same animals can be compared, it is necessary to have a tuberculin at the disposal of the observers which can be relied upon to yield as satisfactory results as can be obtained with such a biological product. For some few years the purified protein derivative (P.P.D.) has been largely used in this country. Its great advantage is that it consists of tuberculo-protein produced during the growth of the tubercle bacillus in synthetically-prepared medium and that it is at least 90 per cent. pure, the remaining 10 per cent. being composed of materials which, so far as practical purposes are concerned, seem to have little or no effects on the results following the intradermal injection of the material. Because of the method of preparation and the various tests, including those for standardisation purposes, the various batches of tuberculin can be made of similar potency: thus the operator can, with confidence, compare the results of tests carried out on the same animals, although considerable intervals of time may elapse between the application of the tests. This is of much importance in herd testings when results of tuberculin tests over a period are necessary and especially when indefinite reactions occur and it is necessary to make retests after an interval in order to arrive at a conclusion on the cause of the reaction.

Tuberculin-testing is carried out in this country on lines somewhat different from those in most other countries. Because of the prevalence, especially in some herds and in some districts, of cattle which are sensitive to tuberculin from allergy developed from agents other than the bovine tubercle bacillus, tuberculin-testing is not such a simple proposition as was at one time thought. With the use of mammalian tuberculin of a potency higher than was used in the past—and a reasonably high potency is necessary in order to elicit reactions in cattle of low sensitivity from bovine tuberculous infection—the incidence of reactions

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associated with agents other than bovine tubercle bacilli has increased. It is true that such sensitivities are not so persistent as are those due to bovine tuberculous infection and that, with time, they become less marked: yet, at some stages, they may be of such a nature as to present considerable difficulty in the interpretation of their significance. In order to assist in the interpretation of the results of tuberculin tests, the " comparative tuberculin test " was introduced into tuberculin-testing some years ago in this country and is now in common use. In this test two tuberculins are used—prepared from the human tubercle bacillus (mammalian tuberculin) and the avian tubercle bacillus (avian tuberculin), respectively. Although there is a considerable overlapping in the response in cattle sensitive to tuberculin from the various causes, experience has shown how the results of the use of these tuberculins can indicate with great accuracy whether or not the sensitivity is caused by bovine tuberculous infection: this is especially evidence in the testing of herds of cattle, the tuberculous history of which is known over a period.

By the method described in the above-mentioned article " tuberculins " can be prepared from the various micro-organisms of the acid-fast group and their activity can be assessed and standardised, both chemically and biologically, with an accuracy of a degree sufficient to ensure sufficient similarity in the different batches of each product. With such similar products it is possible to make comparisons of their value for testing purposes and their relationship, one to another. It is of much interest to read that it is now possible to obtain P.P.D. from the bovine strain of tubercle bacillus in larger quantities than formerly appeared possible; and this was accomplished by laboratory experimentation.

We are still faced with the problem, however, of the overlapping of the sensitivities produced by the various agents in cattle and the consequent responses to the P.P.D.s of the different acid-fast organisms. Reference is made in the article to the research work carried out in an attempt to prepare tuberculins of greater specificity. The aim must be to find methods of treatment of tuberculins whereby the special specific elements in them can be produced in quantities sufficient to obscure the non-specific parts in the amounts injected into cattle for testing purposes. When such products can be produced, tuberculin-testing will become a simpler operation and the interpretation of results will present less difficulty. In the study of the specificity of tuberculins and the research work into the methods for the preparation of products of more specificity, we have an example of the application of science and scientific procedure to a veterinary problem which, when the results are available, should have a practical application to one of the everyday bits of work in the life of many members of the profession.

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GENERAL ARTICLES

WEYBRIDGE P.P.D. TUBERCULINS

By H. H. GREEN,

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General.—It is now well over half a century since Koch first prepared a product capable of eliciting allergic reactions in tuberculous subjects. Koch's original tuberculin was made by growing the tubercle bacillus in glycerinated meat broth for about six weeks, boiling to kill the organism, evaporating to one-tenth of the original volume and filtering through a diatomaceous candle. This gave a viscid brownish fluid, free from bacterial cells but containing residual constituents of the medium, metabolic products produced during growth of the bacilli, and sufficient glycerine (about 40 per cent.) to act as preservative. Koch also prepared other tuberculins in various ways by grinding and extracting the bacilli, but the term "O.T." or "Old Tuberculin" refers to products prepared by the evaporation procedure and is sometimes still used if that procedure is followed, even if a synthetic medium replaces the original glycerinated broth. The tuberculin used by the medical profession in this country is a "heat concentrated synthetic medium" product sometimes referred to as H.C.S.M. and sometimes as O.T.

The Bureau of Animal Industry in the United States of America uses an H.C.S.M. product, grown from three human strains of *M. tuberculosis* on a medium of high glycerine and asparagine content to ensure high yield of active principle. Since Weybridge used the same technique until April, 1942, and still employs portions of it in producing the P.P.D. tuberculin issued since that date, the method of preparation may be given in sufficient detail to guide other workers.

B.A.I. Tuberculin.—Three seeding strains of *M. tuberculosis*, human type, isolated by the Bureau of Animal Industry and designated D.T., P.N., and C., are maintained in separate cultures on glycerine egg slopes if wanted for transport, and on glycerinated broth for transplantation on to synthetic media as required for routine production of tuberculin. The purity of these is tested at intervals by macroscopic, microscopic and cultural tests, and by animal inoculation.

The propagation medium is a conventional beef broth fortified with 1 per cent. peptone and 0.2 per cent. KH_2PO_4, to which is added 7 per cent. of glycerine (70 gms. per litre or 56 ml. if S.G. is 1.25) before charging 100 ml. aliquots into 300 ml. Erlenmeyer cotton-plugged flasks. After autoclaving, final reaction is pH 7.3. These flasks are inoculated from previous cultures not older than 14 days, and with pellicles completely covering the surface, seeding being at the rate of one scale of pellicle per 100 ml. of medium, transferred with a platinum loop $\frac{1}{8}$ in. in diameter, and gently floated on the surface. Incubator temperature is 37.5 deg. C.

From these propagation flasks pellicles are seeded on to the surface of a synthetic medium containing 0.18 per cent. dipotassium phosphate (C.P. quality $K_2HPO_4.3H_2O$), 0.09 per cent. sodium citrate, 0.15 per cent. magnesium sulphate (crystals), 0.03 per cent. ferric citrate (scales), 1.4 per cent. asparagine, 1.0 per

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cent. glucose, and 10 per cent. (by weight) of glycerine. Each of the salts is dissolved separately in distilled water and, in the order given, stirred into the hot asparagine solution made up in half the total final volume. The glucose and glycerine are then added and the final volume adjusted with water. The solution is clear and no filtration is required. The pH value is 7.0 and no adjustment is necessary. Portions of 100 ml. are charged into 300 ml. flasks, as in the case of the propagation media, and autoclaved at 11-12 lbs. pressure for 25 minutes. The cotton wool plugs are covered with tin foil to reduce evaporation during the subsequent prolonged period of growth after inoculation.

The seeding flake grows as a thin film over the surface of the liquid medium in the course of about ten days, and then thickens until, after about eight weeks, the medium is exhausted. The most active growing period is about the fifth week, at which time there is marked condensation of moisture on the upper parts of the flasks. Final growth corresponds to about 2 gms. of bacterial dry weight per 100 ml. of medium. If any of the thickening films sink, growth ceases and yield is lower. The final reaction is well above pH 7.

After rejection of possibly contaminated cultures, an approximately equal number of flasks seeded from each of the three strains D.T., P.N. and C. is assembled and subjected to flowing steam for three hours to kill the organisms. After cooling overnight, the contents of the flasks are strained through 60-mesh copper gauze. Each flask is finally rinsed with 10 ml. of distilled water and the combined rinsings poured through the collected pressed mass of bacterial debris. To the entire strained liquid 1.5 per cent. of glycerine (1.2 ml. per 100 ml.) is added and the product evaporated to slightly less than one-fifth of its volume on a steam bath in corrosion-proof pans, on to the surfaces of which are directed air blasts to reduce evaporation time and changes. The concentrated product is then cooled in suitable flasks, accurately adjusted to one-fifth of original volume, and diluted with an equal quantity of 1 per cent. phenol. The phenolised product is stored at 47 deg. C. for a fortnight to ensure permanent limpidity of the finished product, and passed through a Sharples high-speed bowl-type centrifuge two or three times to ensure subsequent ease of passage through sterile diatomaceous filters into sterilised containers, under air pressure of about 8 lbs.

At this stage the phenol content is checked and an " acetic acid test " applied to indicate the content of active principle in the finished product. In this test 15 drops of glacial acetic acid are added to 20 ml. of tuberculin in a graduated tube. After standing 48 hours the precipitate should show a volume of about 5 ml.

Batches are tested for safety and potency on normal and sensitised guinea-pigs, and bottling is conducted under aseptic conditions. A tentative expiry date of one year is assigned on the labels. Sample bottles are checked for absence of micro-organisms by cultural tests and microscopic examination.

The potency aimed at in the B.A.I. procedure was originally that of a " standard Koch's O.T.," i.e., it substituted a synthetic medium for the old meat broth medium, so providing a cleaner product in which all the more complex organic constituents were derived from the organism itself. It still, however, contains the concentrated decomposition residues of the synthetic medium and unwanted metabolic by-products of bacterial growth, besides the active principle itself.

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Purified Protein Derivative.—As early as 1891 Koch believed that the substance responsible for eliciting the specific effects in the diseased animal was of protein nature, and the literature on "The Chemistry of the Proteins of the Acid-Fast Bacilli" over the ensuing half-century was reviewed by Florence Seibert in *Bacteriological Reviews*, March, 1941. Seibert herself has done much to elucidate the nature of the active fractions of tuberculin and the term "P.P.D." (purified protein derivative) was coined by her about 1934 to replace one of her earlier T.P.T. products. As early as 1926 she isolated a crystalline protein with tuberculin activity, although this constituted only a portion of the total active protein. In 1932 she prepared a trichloracetic precipitated tuberculin protein suitable for the preparation of a standard tuberculin, having already shown that similar products could be obtained by a variety of precipitation procedures. Her earlier preparations were generally made from H.C.S.M. tuberculin by ultra-filtration through collodion impregnated alundum candles, "washing in solution" to remove dialysable material, and precipitating the residual solution (containing the protein, some polysaccharide and nucleic acid) with trichloracetic acid. The precipitate, after washing with anhydrous ether, yielded a dry powder stable for years and suitable for making up solutions on a *precise quantitative basis.* This product still contained considerable quantities of nucleic acid (up to 9 per cent.) and polysaccharide (5 per cent. or more) and gave all the specific reactions of tuberculin. Tuberculin filtrates freed from protein did not give the required specific reactions. At a later date she reverted to the use of saturated ammonium sulphate (buffered at pH 7.0 with disodium phosphate) in order to reduce the nucleic acid content of the powder, and in 1941 published her later methods of preparation and analysis of a large quantity of P.P.D. for use as standard. The method retained the somewhat cumbersome collodion ultra-filtration, and final drying was effected from the frozen state instead of by ether. The product contained much less nucleic acid but about the same amount of polysaccharide as before. It was claimed to be less "denatured" and about twice as potent as her 1934 product, but there is room for serious doubt on this point since the statement rests on inadequate comparisons on the human subject, and no adequate "serial titrations" seem to have been made on experimental animals. In comparisons of potency by biological testing it should be remembered that even an 8 "guinea-pig test," using four dilutions on each side (1/500, 1/1,000, 1/2,000, 1/4,000 of O.T. "potency equivalent") is still susceptible to an error of ±40 per cent., and that it takes statistical analysis of data from 50 guinea-pigs to reduce the error of comparison below ±10 per cent.

Seibert's "Reserve Standard P.P.D." has been extensively used in the United States on the human subject, and since it probably has almost maximum obtainable potency as well as experimental priority it might well become a provisional "international standard" for P.P.D.s prepared from human strain *M. tuberculosis.* Complications arising when "comparative testing" is carried out with mammalian and avian strains need not be discussed at this juncture.

Whilst Seibert's work was going on in America, satisfactory P.P.D.s were also made in other countries by similar techniques; by Doig *et al* in England in 1938, by Jensen in Denmark in 1938, by Wong and Chu in China in 1938, by Vásárhelyi and Gözsy in Hungary in 1939, and by Leyva in the Philippines in 1940.

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The Veterinary Laboratory of the Ministry of Agriculture at Weybridge also interested itself in the question in 1938 and prepared a few specimens of P.P.D. by Seibert's original technique from its own H.C.S.M. tuberculin, made along the lines of the B.A.I. tuberculin since 1935. These were originally intended to serve for biological standardisation of the official H.C.S.M. issues, but it soon became apparent that a much simplified technique could be adopted for routine manufacture of P.P.D. tuberculins for veterinary use, and, after appropriate large-scale testing on cattle throughout the country, P.P.D. tuberculin of both mammalian and avian origin were adopted for official use early in 1942. When studying the earlier preparations it was noted that the cumbersome collodion ultrafiltration (selected by Seibert in her first work on crystallisation of tuberculin protein, and adhered to in her own later work) could be dispensed with, and that direct precipitation with trichloracetic acid, followed by centrifugal washing to remove media constituents and soluble metabolic products, could be substituted. Preparations made by W. M. Allcroft in 1938, comparing P.P.D.s made by the ultrafiltration and centrifugal washing techniques, gave identical potency values when tested by J. Reid on sensitised guinea-pigs. Re-solution in phosphate buffer and second precipitation for further purification made no material difference. Collodion filtrates from H.C.S.M. tuberculin gave no precipitate with trichloracetic acid and, in the high dilutions used for guinea-pig testing, gave no skin reaction. When P.P.D.s, made by the prevailing Seibert technique, and by direct precipitation and centrifugal washing, followed by anhydrous ether drying, were compared with the H.C.S.M. tuberculin from which they were made, in solutions of equivalent protein content (as determined by chemical analysis), results on guinea-pigs were apparently identical in all three cases. Since these observations fitted theoretical expectation, the traditional ultrafiltration washing was omitted in later work, which at this stage was of desultory character in any case.

The following year a study of chemical changes occurring during the growth of acid-fast organisms was made by A. Eden (and the writer) and losses of protein during heat concentration of tuberculin were determined. These were surprisingly large ($\frac{1}{3}$ to $\frac{1}{2}$ of total, by surface denaturation to insoluble form during air-blast evaporation) and it became apparent that much larger yields of P.P.D. could be obtained by direct precipitation of steamed bacterial filtrates. The specificity reactions of a series of P.P.D.s from six different acid-fast organisms prepared by Eden in this way was partially investigated by Reid in 1940, and more extensive work by J. L. McGirr in 1942 is recorded below. One of Eden's preparations was used as tentative standardising agent for H.C.S.M. issues for two years, later replaced by one of McGirr's preparations after routine P.P.D. tuberculins were officially issued. It was found that chemical determinations of protein in H.C.S.M. issues served as accurate measures of potency, and analyses of stored samples of earlier batches gave a more precise record of previously used field material than biological testing had done. The batches of mammalian H.C.S.M. used in certain "comparative tests" in cattle under field conditions happened to contain 1.5 mg. of tuberculin protein per ml. at a time when corresponding avian H.C.S.M. contained 0.4 mg. per ml. and since an arbitrary system of interpretation of "comparative reactions" had been devised for distinguishing between "specific" and "non-specific" infections, these potencies were selected for official issues of "P.P.D. Tuberculins" in 1942.

They are not final, but have been used satisfactorily for four years on the basis of interpretation devised by J. N. Ritchie and his colleagues on the Inspectorate Staff of the Ministry. The " comparative test " is far from infallible in individual cases, but is regarded as a valuable group test when something is known of the previous history of the herd, and is sufficiently good to characterise the nature of new infections in previously tested herds.

Manufacture of Weybridge P.P.D. Tuberculins.—The method of manufacture follows that described above for B.A.I. tuberculin up to the point at which the culture flasks are steamed, except that instead of using 100 ml. quantities of synthetic medium in 300 ml. Erlenmeyers, 1,000 ml. quantities are used in flat " penicillin flasks " (autoclaved at 15 lbs. for 8 minutes), and the growth period is extended to 10 weeks. Several flakes of pellicle from the propagation media are used instead of one, to ensure uniform rapid growth of the surface film, and the metal foil covering of the cotton plugs is omitted, partly to ensure better æration and partly because evaporative losses are relatively lower for the larger volumes concerned. The yield of P.P.D. per 100 ml. is the same as for the B.A.I. flasks, but economy is effected in incubator space and in various manipulative procedures. Still larger culture flasks can be used, but difficulties may be encountered with sinking growths and accidental contaminations may involve greater wastage.

After steaming the cultures, straining through copper gauze and discarding the rinsed bacterial debris, the liquid is sucked through a ½" pad of pressed filter-paper pulp on a Buchner funnel to remove residual cellular material and provide a perfectly clear filtrate. This filtrate is transferred to tall litre measuring-cylinders and one ninth of its volume of 40 per cent. trichloracetic acid solution is added to bring the whole to 4 per cent. concentration. Total protein is precipitated, allowed to sediment overnight in the cold store, and the supernatant solution (containing unwanted residues of the medium and soluble bye-products of bacterial growth) is syphoned or sucked off. The sedimented sludge is rinsed into 250 ml. centrifuge tubes, with dilute trichloracetic acid (1 per cent.), the amount derived from two or three cylinders going into one tube, and centrifuged for 10 minutes to remove residual supernatant. The compact protein centrifugate is then resuspended in 200 ml. dilute trichloracetic acid to wash out media residues, centrifuged for 15 minutes, the supernatant again discarded and the precipitate resuspended in 200 ml. of 0.3 per cent. KH_2PO_4 solution (i.e., lightly buffered water) to wash out the trichloracetic acid. Centrifugation at this washing takes half an hour at 2,000 r.p.m. before a clear supernatant is obtained. After discarding this, the washed purified protein derivative may be taken to the dry state after washing with acetone and ether, but better dry preparations are obtained by the modified technique described below, and for practical issues of tuberculin the wet precipitate is simply dissolved up in M/15 Na_2HPO_4 solution to provide a concentrate suitable for preservation in cold store until required for further dilution to issue strength. Because of the buffering effect of the trichloracetic precipitated protein, the reaction of the redissolved " crude P.P.D." is too acid, and sufficient M/15 NaOH is added to bring to about pH. 6.8. A solution of 50 per cent. glycerol, 2.5 per cent. phenol, and 2.5 per cent. sodium chloride is then added in amount sufficient to bring the final concentration, at about 1/10th

of the original culture filtrate volume, to 10 per cent. glycerol, 0.5 per cent. phenol, and 0.5 per cent. sodium chloride. The pH. is finally adjusted electrometrically to 7.0.

This neutral concentrated solution contains the true P.P.D. of low molecular weight, but also contains 5-10 per cent. of its total nitrogen in the form of higher molecular weight material of lower potency, which clogs the final Seitz filtration prior to bottling. The latter is therefore removed by passing three times through an air-driven Sharples supercentrifuge running at 35,000 r.p.m. and delivering about 5 litres per hour. The unwanted debris adheres to the rotating bowl and is removed after the first run, although not necessarily between the second and third runs.

The protein nitrogen of the centrifuged concentrate is then determined, and this multiplied by 7 (or whatever factor is given by the reserve standard dry P.P.D. used for final biological standardisation) is taken as representing the P.P.D. content. The concentrate averages about 1 per cent. protein, and, being stable more or less indefinitely in cold store, is kept until required for dilution to issue strength of 1.5 mg. per ml. The diluting fluid is a solution of 0.5 per cent. phenol, 0.5 per cent. sodium chloride, and 10 per cent. glycerol in M/15 phosphate buffer adjusted to pH. 7.0. The finished " P.P.D. tuberculin " is then passed through 20 x 20 cm. Ford Sterilmats (G.S. grade) in a filter press under a few lbs. air pressure, into sterile containers, and bottled under aseptic conditions in 1 ml., 3 ml., and 5 ml. phials. In making the final dilution a small allowance is made for adsorption of protein in filtration (which amounts to about 2 per cent. of the total when 50 litres go through four sterilmats), such that the bottled material shows the accurate figure of 1.5 mg. P.P.D. per ml. The phenol concentration of 0.5 per cent. is also determined by the Folin colour reaction as an additional check against the operations of the various technical assistants who perform the routine work. The usual tests for absence of micro-organisms are conducted on sample phials, and biological tests are carried out on sensitised guinea-pigs and on cattle. Although the latter are really unnecessary, since the product is always made in the same way from the same strains, and the analytical check on quantity of protein is accurate to $\pm$ 1 per cent., there is always the remote possibility that a potency variation may occur, and no veterinarian would be satisfied without a biological cross-check.

In making the guinea-pig test, 8 albino animals in good condition and of weight over 350 gms. are sensitised by intramuscular injection of 0.5 mg. (wet weight) of live organisms, and three weeks later each receives four intradermal injections of 0.2 ml. tuberculin, at dilutions of 1/500, 1/1,000, 1/2,000, 1/4,000 on one side of the clipped depilated skin parallel to the vertebral column. On the opposite side at similar points four similar injections are given of a standard preparation made from the reserve stock dry P.P.D. powder described below. Readings are taken at the 24th and 40th hours and calculations of relative potency are made. In no single case, over four years' issues, has the biological test contradicted the chemical test, and no variations between batches of tuberculin have occurred. The 8 guinea-pig test has an accuracy of $\pm$ 40 per cent., and therefore fails to reveal differences of lesser magnitude in material already stand-

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ardised to ± 1 per cent. by chemical analysis, but occasional 50 guinea-pig tests subjected to statistical analysis show that the larger the number of animals used the closer do the biological potency tests approximate to the analytical tests.

The test on not less than 8 cattle in an infected herd under field conditions is in no sense a true standardisation, since the error in animals of naturally varying allergic status is too great, but it serves to reassure the farming public that "no difference between successive issues can be detected." For the cattle test serial dilutions varying from 1/10 down to 1/200 of full strength are compared with similar concentrations of freshly made standard P.P.D. at comparable sites on opposite sides of the neck in 0.1 ml. intradermal doses. If the tuberculous cattle do not display a suitable allergic status the test is repeated on others.

Much trouble in animal testing can be avoided by pooling P.P.D. concentrates kept in cold storage from successive incubations and treating the enlarged stock as a single batch, before standardisation and bottling. Alternatively, reserves of dry powder (acetone-ether dried or freeze-dried) can be accumulated, standardised, and reconstituted to issue strength as required.

It may be added that the traditional M/15 concentration of buffer phosphate used in the diluting fluid is unnecessarily strong, and that it is intended to reduce this to M/75. The 10 per cent. glycerol is an arbitrary addition (following B.A.I. precedent), and does not materially improve keeping qualities.

It is also intended to distinguish avian tuberculin (below) from mammalian tuberculin by colouring it red. The phials are already distinguished by red and blue labels, but a coloured fluid in the inoculating syringes should be a useful precaution against transposition.

Preparation of Dry P.P.D. Powder.—This is best prepared from the concentrate after removing alien material by the Sharples supercentrifuge, reprecipitating with trichloracetic acid, washing once or twice with water (prolonged centrifuging at 2,000 r.p.m.), twice with small quantities of anhydrous acetone to remove water, and then with anhydrous ether as often as required to bring the stirred material to the fine, flakey state, which, on removal of ether and drying in a vacuum dessicator, crumbles to a soft, light, nearly white powder. This, especially if stored over phosphorus pentoxide to keep quite dry, retains its potency and specificity for an indefinite number of years. A moderate reserve stock (80 gms.) of this, prepared by J. L. McGirr, has served as standard since 1942.

For larger quantities freeze-drying is probably preferable.

A powder prepared in this way still contains about 7 per cent. of nucleic acid and 5 per cent. of polysaccharide, and is therefore only about 88 per cent. true protein. But both of the alien constituents are regarded as inert from the point of view of the intradermal reaction, and it has not been considered necessary to purify ordinary P.P.D. any further.

It is of interest to record, however, that of about 40 different products prepared from acid-fast organisms, one human strain of highly purified P.P.D. prepared by reprecipitation with metaphosphoric acid to free from polysaccharide, then salted out with ammonium sulphate at pH. 7.2 to remove nucleic acid, and finally brought back to original form by reprecipitation with trichloracetic acid,

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washing, and ether drying, showed only 1 per cent. each of nucleic acid and polysaccharide. This product (recorded W.H.15) of 98 per cent. true protein content is, from the chemical point of view, probably the purest ever prepared anywhere. Its potency and specificity, however, as tested on 8 guinea-pigs sensitised to human strain and 8 sensitised to avian strain, did not differ significantly from the less pure reserve standard P.P.D. from which it was derived. Such guinea-pig tests, with their error of ± 40 per cent., could not be expected to distinguish between 88 per cent. and 98 per cent. purity, but they do indicate that potency is not adversely affected by serial reprecipitations.

A number of specimens of dry P.P.D. have been prepared from 5 different laboratory strains, including the 3 B.A.I. strains, P.N., D.T. and C., the well-known H.37, and a Weybridge strain. Although the rate of growth and yield of P.P.D. obtained per litre of medium varied considerably, no differences could be detected in potency or specificity of the final dried powders.

Avian P.P.D. Tuberculin.—The procedure is the same as for mammalian tuberculin, except that the final issue strength is 0.4 mg. per ml. (quarter) for reasons given above.

Three strains are again used, Dn., 485, and 7169, although the P.P.D.s prepared from each do not differ and the continuation of an older practice is traditional rather than logical. Although growth is good, the natural yields of P.P.D. from avian strains are rather less than one third of those obtained from human strains. There is very little high molecular weight debris to be taken out during centrifugation in the Sharples supercentrifuge.

Potency tests are carried out on guinea-pigs (8) sensitised with an avian strain of *M. tuberculosis,* but only in exceptional circumstances, for special reasons, on cattle known to be " non-specific " reactors.

Dry avian P.P.D. powder is prepared in the same way as human strain P.P.D. The stock provisional standard still contains 6.7 per cent. nucleic acid and 4.4 per cent. polysaccharide. It has a total nitrogen content of 14.4 per cent., and the conversion factor in Kjeldahl determinations is therefore approximately 7. A specimen more highly purified by reprecipitation with metaphosphoric acid, followed by salting out with ammonium sulphate, showed only 0.7 per cent. of nucleic acid and 0.6 per cent. of carbohydrate (T.N. 15.6 per cent.), but did not differ significantly in potency or specificity from the ordinary standard.

Bovine P.P.D.—The strains used are A.N.5 and C.45. The method of preparation is practically the same, except that bovine strains are less tolerant of glycerine than human strains, and therefore grown on the modified Sauton medium of Watson and Reid, which has the composition: Asparagine 0.5 per cent., glucose 1 per cent., glycerol 6 per cent., magnesium sulphate 0.1 per cent., potassium dihydrogen phosphate 0.2 per cent., ammonium citrate 0.2 per cent., sodium chloride 0.2 per cent., ferric ammonium citrate 0.0075 per cent., the whole being brought to about pH. 7.3 with ammonia.

The yield of P.P.D. under these conditions is only about one fifth of that obtained from human strains, and this appears to be one of the reasons why human strains have been continued for the so-called " mammalian tuberculin " used in cattle testing. Theoretically, bovine strains should be better.

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A laboratory bovine strain, however, has been educated at Weybridge to grow at higher concentrations of glycerine, and now grows freely on the B.A.I. medium used for human strains. Under these conditions the yield of P.P.D. rises to about that of human strains, and the somewhat more specific bovine product could therefore be issued just as easily if desired. At present there is not sufficient evidence to justify deviation from conventional practice in most parts of the world.

Other P.P.D. Preparations.—Purified protein derivatives have also been prepared from other acid-fasts for experimental purposes, including B.C.G., *M. johnei*, and the non-pathogenic *M. phlei*. Certain "C.T.P.D." preparations ("chemically treated protein derivatives," altered in such a way as to damage certain molecular groupings more than others and so alter specific characters) have also been made, but since their practical value for field use has not been properly investigated they need not be discussed here.

With regard to the specificity characters of the six P.P.D.s mentioned above, the following table is an amplification by J. L. McGirr in 1943 of preliminary observations by J. Reid in 1940.

Defining "specificity factor" as the number of weight units of heterologous P.P.D. required to elicit the same intensity of skin reaction as one unit of homologous P.P.D., and using 40 guinea-pigs in groups of 8 for each type of sensitisation (240 animals in all), it has proved possible to assign numbers to each P.P.D., representing specificity relationships.

Chart of Specificity Factors.

Type of Sensitisation.	P.P.D. Human.	Bovine.	Avian.	Johne.	Phlei.	B.C.G.
Phlei ...	150	150	100	50	1	150
Human ...	1	½	20	30	150	2
Bovine ...	1	1	40	30	150	2
Avian ...	20	40	1	3	100	40
Johne ...	10	10	3	1	50	10
B.C.G. ...	1	½	20	30	150	1

In obtaining these numbers the guinea-pigs were sensitised in the usual way with live organisms in the case of human, bovine, and avian strains. In the case of Johne's bacillus and *M. phlei*, non-infective to guinea-pigs, sensitisation was carried out by injections of dried organisms in oily suspension.

It will be noted that all six P.P.D.'s show cross reactions, although it takes 150 units of phlein P.P.D. to elicit the same reaction as 1 unit of any of the mammalian P.P.D.s when the animals are sensitised by mammalian organisms. The mammalian P.P.D.s fall into one group with small differences in specificity factors, although it is of interest to note that bovine P.P.D. is peculiar in eliciting a greater reaction in human sensitised guinea-pigs than does the homologous human P.P.D. In this connection the observation of Schaffer in 1942 may be recalled. He claims to have detected two different antibodies to specific tubercle protein, in guinea-pigs infected with a "smooth" bovine strain. One of these appeared to be specific for bovine organisms, while the other seemed common to

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both human and bovine types. In his experience the human strain produced only the latter antibody.

It will also be noted from the table that twice as much avian P.P.D. is required to elicit the same reaction in bovine sensitisations as in human. This suggests that bovine P.P.D. might be more useful in the "comparative test" for distinguishing between specific and non-specific infections in cattle. The point has not yet been tested.

It will also be noted that although the specific difference between avian and johnin P.P.D. and mammalian P.P.D.s is quite wide (the basis of the "comparative test"), the difference between avian and johnin P.P.D.s *inter se* is quite small. It only takes 3 units of johnin to match 1 unit of avian P.P.D. in avian sensitised guinea-pigs, or 3 units of avian P.P.D. to match 1 unit of johnin in johne sensitised guinea-pigs. Limited tests on cattle suggest that the difference is even smaller with that species, and much the same reactions are produced by both P.P.D.s in non-specific infections. Hence a johnin test adds nothing to the information gained in the ordinary comparative test using mammalian and avian P.P.D.s. If the "comparative test" shows an infection to be non-mammalian, other means than tuberculin testing have to be adopted to determine whether that infection corresponds to Johne's disease, or to harmless sensitisation with poultry strains, or to other obscure acid-fast sensitisation.

Nature of P.P.D.—Although it has been conclusively demonstrated that the active principles in tuberculins are protein in character, and that these are of small molecular size, it can be shown by physico-chemical means that any given P.P.D. is a mixture of several proteins with similar biological properties. Attempts to separate these by salting-out procedures, or by ultra-centrifuge techniques, may yield products of different molecular size, but when tested for potency and specificity on a gravimetric basis all show the same properties as allergens in the intradermal reaction. All the evidence points to the existence of a primary biological unit of molecular weight in the region of 8,000. Two of these units linked loosely together, or physically aggregated, give a molecular size of 16,000 and four a size of 32,000. Any products reduced below the smallest unit by enzymic action lose their biological activity altogether. Any larger molecules give the tuberculin reaction although those of medium small size are not antigenic, in the sense that they do not sensitise the skin of a non-reactor, to the alergic reaction they are capable of eliciting in a naturally infected animal. They can, therefore, be used in re-testing an animal at appropriate intervals, without danger of an earlier test seriously interfering with the reactions in a later test. Very large molecules of tuberculo-proteins, as they occur in the organism itself, do sensitise to the intradermal reaction which the smaller partially denatured molecules of a steamed culture filtrate are still capable of eliciting. A raw filtrate from media in which live organisms are growing contains a small proportion of heat-coagulable protein of higher molecular weight which is entangled with the bacterial cells on steaming the culture, and is filtered off through the filter-paper pulp in the manufacturing process outlined above. Only the water-soluble non-coagulable proteins of small size pass into the clear filtrate, to be precipitated by the trichloracetic acid. When

the precipitate is redissolved in phosphate buffer a small proportion of less soluble, higher molecular weight material, either originally present or formed by aggregation of smaller molecules, is present in the concentrate, but this is taken out by the Sharples Supercentrifuge. The final P.P.D. consists of a mixture of small non-antigenic molecules in uncertain proportions, probably ranging from the primary chemical unit to complexes about eight times as big—say, 8,000 to 64,000, with the majority about 16,000 in molecular weight. All these have exceedingly similar biological properties, so that the mixture can be regarded as a single *biological entity* although not as a single chemical entity. Specific and non-specific characters are, therefore, attributable to different chemical groupings or biological patterns in the smallest primary unit, and not to different protein molecules. The fact that mammalian P.P.D. gives a lesser non-specific reaction in avian infections, and avian P.P.D. gives a lesser non-specific reaction in bovine infections, is not due to separate proteins in the same P.P.D. but to separate chemical groupings in the same primary molecule. The primary molecules in P.P.D.s made from different acid-fasts are different, each with its own structural groupings of atoms, but the state of aggregation or loose combination of smaller molecules into larger ones makes no difference within the limits of size actually present. There is, therefore, no hope of separating specific from non-specific components in any given P.P.D. There is, however, hope that the specific and non-specific assemblies, in the constellation of atoms which constitute the primary biological unit, may be differentially attacked by chemical means in such a way as to enhance specificity. This is the direction of research followed by J. L. McGirr at Weybridge in 1943-1944, and which it is hoped to extend further as opportunity permits. Treatment of mammalian P.P.D. (either human or bovine) with ninhydrin or chloramine T, or by exposure to strong light in dilute solutions, or by gentle oxidation procedures, damages the non-specific groupings much more than the specific groupings and therefore yields a C.T.P.D. *more specific* than the P.P.D. from which it was derived. The same type of treatment applied to an avian or johnin P.P.D. damages the specific groupings more than the non-specific ones and yields a C.T.P.D. *less specific* than the mother protein.

Chloramin-T treated human strain P.P.D. gives much the same reactions as the mother substance in guinea-pigs sensitised with human strain *M. tuberculosis,* but in guinea-pigs sensitised with avian strain the reactions are very different. The "specificity factor" recorded as 20 in the table above is raised to 80, without significant reduction in "potency" so far as human strain sensitisation of guinea-pigs is concerned. When used on tuberculous cattle in the field, however, erratic results are encountered and practical applications, therefore, await further research. There is, apparently, a pronounced difference in different species of animals—an interesting biological point which may dethrone the guinea-pig from its hitherto proud position in tuberculin research. The data of J. L. McGirr on over 2,000 guinea-pigs and a moderate number of bovine subjects was submitted to the Veterinary Educational Trust in 1944, in terms of his Wellcome Research Fellowship, as a report on "The Specificity of P.P.D. Tuberculins," but, for various reasons, completion for publication has been delayed.

Relative potencies of officially recognised tuberculins.—Although P.P.D. tuberculins are cleaner products which can be issued at precise strength on a

gravimetric basis, or as analytically checked solutions of any selected potency, and are, therefore, preferable to H.C.S.M. products standardised only on a biological basis with a much larger margin of error (±40 per cent. in an 8 guinea-pig test as compared with ±0.5 per cent. on a chemical analysis), the real difference in practical use at high dilutions in the Mantoux test in the human subject, or at neat O.T. strength in the intradermal test on cattle, is not very great. Because of the removal of evaporated media constituents and alien metabolic products of bacterial growth from O.T. types of tuberculins, P.P.D. tuberculins tend to give more uniform reactions in tuberculous subjects, but the practical difference in diagnosis is not great and there is no fundamental objection to continued use of O.T. tuberculins. It is important, however, that relative potencies be clearly recognised. A sample of "provisional O.T. standard," procured from the M.R.C. Laboratories at Hampstead and analysed at Weybridge, showed a protein content of 2 mg. per ml., and on testing on guinea-pigs against Weybridge P.P.D. it matched a solution made up at 2 mg. per ml. For reasons already given, the issue strength of Weybridge mammalian P.P.D. tuberculin has been 1.5 mg. per ml. over the last four years, i.e., ¾ O.T. potency. This concentration, however, originally fixed for the "double intradermal test" on cattle, using a dosage of 0.1 ml. at the neck site, in distinction to the B.A.I. single intradermal use of 0.05 ml. in the caudal fold, is not necessarily final and may be altered if the single intradermal test is introduced for attested herds.

Three samples of B.A.I. tuberculin procured from the United States showed, on analysis at Weybridge, 0.22 mg., 0.25 mg., and 0.28 mg. protein per ml., and on comparison with solutions of Weybridge P.P.D. (made up in glycerinated phenolised buffer phosphate) in guinea-pig tests showed potencies equivalent to their protein contents. The B.A.I. product therefore appears to be somewhat stronger than the Hampstead O.T. used for standardising commercially prepared O.T. tuberculin for medical use in Great Britain.

No opportunity has yet occurred of comparing the 1941 P.P.D. of Seibert and Glen with the 1942 Weybridge "reserve P.P.D." in respect of potency and specificity, but there is no reason to believe that they are significantly different, and in any case, Seibert's stock could probably be treated as a useful international standard for all tuberculins derived from human strains.

In conclusion, it may be mentioned that because of the high concentration of P.P.D. used in veterinary practice as compared with medical practice (0.15 mg. per dose, as against 0.00002 mg. to 0.005 mg. per dose), and the fact that American P.P.D. is used for the human subject but not for cattle, the production of P.P.D. at the Weybridge laboratory probably exceeds that of any country in the world. Present issues exceed 60 litres (600,000 doses) of each tuberculin (mammalian and avian) per month, and will probably exceed ten million doses of each for the year 1947. If used in medical practice at the 0.00002 mg. American test dose, or the 0.005 mg. dose for epidemiological work, or at the dilutions of one per thousand to one in ten thousand O.T. used in Britain, Weybridge issues of human strain P.P.D. would suffice for many thousands of millions of individual intradermal injections per annum. A very small diversion of veterinary issues for medical use would cover all human requirements in Britain.

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VIBRIO FŒTUS AGGLUTININS IN BOVINE SERA

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Vibrio fœtus infection in cattle is not a disease of great economic importance in this country; nevertheless, it is felt that some useful purpose might be served by this addition to the rather scant literature on the subject.

In the Report of the Departmental Committee on Epizootic Abortion (1913) the first brief mention is made of the isolation of vibrios from cases of abortion in cattle, in a herd in Ireland, and in Wales in 1911. The infection in cattle was first recorded in detail by Smith (1918), who isolated the organism from the fœtuses of aborting cows. He reported that lesions in cattle associated with this organism are largely, if not exclusively, restricted to the fœtal membranes, more particularly the chorion, and the fœtus suffers secondarily from a gradually increasing interference with the placental circulation.

Affected cattle usually abort from the fifth to the seventh month of pregnancy, but may abort as early as the second month. The fœtus is generally bloated from the presence of subcutaneous œdematous fluid, and there is excess fluid, generally blood-tinged, in the serous cavities.

Plastridge and Williams (1943), in the United States of America, investigated the incidence of *V. fœtus* titres in infected and non-infected herds, and from their results concluded that a titre of 1 :200 should be regarded as positive, 1 :100 as suspicious, and 1 :50 negative. They state that the agglutination test is of value in diagnosing abortions due to *V. fœtus,* and in determining the probable presence of *V. fœtus* infection in a herd of cattle.

In general, it may be said that cattle are highly resistant to infection with *V. fœtus,* that cases of abortion associated with this organism are sporadic, and that the infection in a herd tends to die out, owing to the fact that the animals quickly overcome the infection, and also to the fact that the organism itself has very low powers of survival.

The source of the infection in cattle remains obscure, but it may be that the reservoir of infection is the sheep, in which animal the disease is more often seen.

The present work was carried out on the blood samples which were received at the laboratory for the routine diagnosis of *Br. abortus* infection. No information was given of the clinical condition of the animals concerned, or of the herd history, and therefore the results comprise merely a record of titres.

Two hundred and fifty-five bovine blood samples were examined in duplicate for *Br. abortus* and *V. fœtus* agglutinins. The *V. fœtus* antigen was prepared from a strain obtained from the National Collection of Type Cultures. The antigen was monovalent, because, as far as we knew at the time, *V. fœtus* seemed to be an antigenically homogeneous species. The strain used was well adapted to artificial growth. It was cultivated on serum agar slopes for three days at 37° C., the growth was suspended in 0.5 *per cent.* saline, standardised by means of Brown and Kirwan's opacity tubes to contain approximately 2,000 million organisms *per* millilitre, and preserved by the addition of 0.5 *per cent.* formalin.

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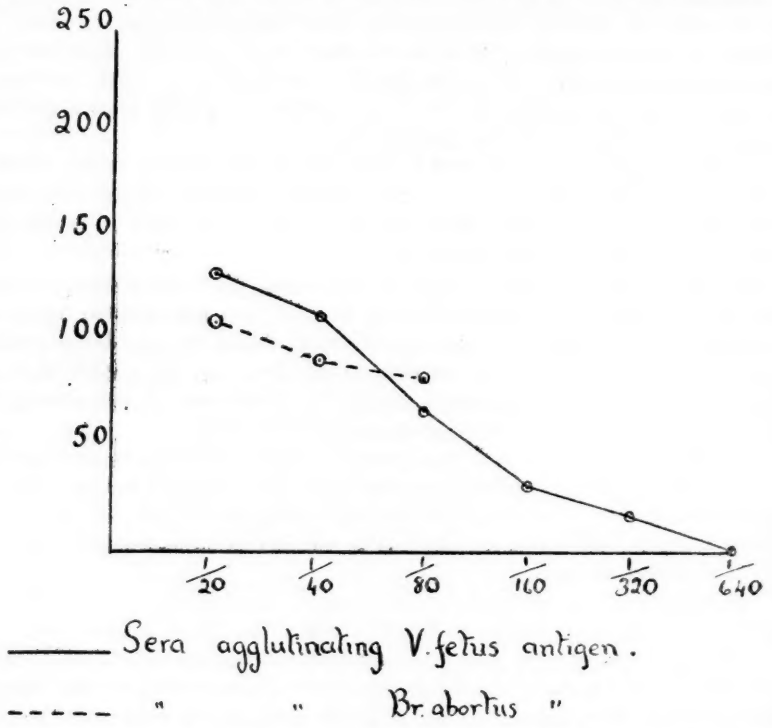
The macroscopic method of testing was used, the tubes being incubated overnight at 37° C. In the case of the *Br. abortus* tests, the dilutions used were 1 :20, 1 :40, and 1 :80; in the case of the *V. fœtus* tests, the dilutions prepared were 1 :20-1 :320. All sera reacting positively at 1 :320 were retested at higher dilutions to determine the titre. (These tests were carried out before Plastridge and Williams' article was published, and therefore the results cannot be correlated exactly with their estimates of the diagnostic titre, 1 :200.) The usual controls were employed for the brucellar abortion tests. For the vibrionic abortion tests, naturally occurring high titre sera were used to test the agglutinability of the antigen, and guinea-pig serum was used to test for the absence of auto-agglutinability of the antigen in normal serum. The guinea-pig serum proved



consistently negative, but whenever any sedimentation of the antigen took place in this control tube, fresh antigen was prepared for use in subsequent tests. It was found convenient to subcultivate the strain of vibrio twice a week on serum agar slopes, and to utilise the fully-grown cultures as antigen, after the subcultures had been made.

The diagram gives the numbers of samples agglutinating at each dilution, with *Br. abortus and V. fœtus* antigens. Of the 255 samples, 93 (36.47 *per cent.*) gave a positive reaction to the agglutination test for *Br. abortus* infection. Eighteen samples (7 *per cent.*) reacted positively to the test for *V. fœtus* infection. (This, of course, is a slight underestimate, since only those reacting to a

titre of 1 :320 have been regarded as definitely positive.) The highest titre recorded was 1 :640—only one sample reached this titre.

Owing to the paucity of details submitted with the samples, it is difficult to draw any significant conclusions from the individual data, but the following observations may be of interest.

(1) In one herd of fifteen animals, vibrionic infection was known to exist, the organism having been demonstrated in the stomach contents and membranes of a fœtus aborted by one of the animals. (There was no sign of *Br. abortus* in the material.) In this herd, only one animal was completely free of blood agglutinins to *V. fœtus*. The remaining fourteen animals showed blood titres ranging from 1 :20 to 1 :80. One may assume that these animals all had contact with the infecting organism. In this herd, too, there were four animals reacting positively to the test for brucellar abortion.

A second herd of fourteen animals tested at the same time formed a useful control. This herd contained three reactors to the *Br. abortus* test, but not one animal showed agglutinins to *V. fœtus*.

(2) Of the seventeen animals known to be bulls, twelve reacted to a titre of 1 :20, ten to 1 :40, six to 1 :80, and one to 1 :160. The higher titres, at least, seem to signify actual infection at some time. It is not known whether this implies a simple, transient blood invasion by *V. fœtus* or an infection of the testicle by the organisms as in brucellar infection of the male animal. Ten of the bulls were positive reactors to the brucellar abortion test.

(3) The sera of thirty-three animals in herds known to be free of brucellosis contained agglutinins to *V. fœtus*. It is reasonable to assume that there had been cases of abortion in some, at least, of these animals, since the blood samples were submitted specifically for examination for *Br. abortus* agglutinins, and further conjecture would lead one to suppose that *V. fœtus* was at the root of the trouble.

(4) From an analysis of the herd data, it seemed that if *V. fœtus* infection existed in a herd, practically every animal in that herd showed evidence of having encountered the organism.

(5) The prozone phenomenon was detected in four of the *V. fœtus* tests. The following titres were recorded in these sera :—

	1 :20	1 :40	1 :80	1 :160	1 :320	1 :640	1 :1,280
2,993/B9 ...	—	+	+	+	—		
2,993/B10 ...	—	+	+	+	—		
2,995/1 ...	—	+	+	+	+	—	
3,704/20 ...	—	+	+	+	+	+	—

From the above results one may conclude that the prozone phenomenon will not interfere with routine tests, since the recommended dilutions of serum for use are 1 :50, 1 :100, 1 :200, and 1 :400.

The prozone phenomenon was recorded only once in the case of the *Br. abortus* tests.

Conclusions

Of 255 bovine blood sera, 7 *per cent.* gave positive reactions to the agglutination test for *V. fœtus* infection : 36 *per cent.* reacted positively to the test for *Br. abortus* infection.

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There is no cross-agglutination between the two organisms.

Brucellosis and *V. fœtus* infection may coexist in a herd. In such a herd, mentioned above, a case of abortion appeared to be caused by the latter organism. This should be kept in mind when the efficiency of vaccination against brucellosis is being evaluated.

It is recommended that the agglutination test for *V. fœtus* infection be carried out as a routine measure in herds in which abortion has occurred, and in which brucellosis and trichomoniasis have not been detected. The test, of course, will not give a definite indication of the cause of any abortions which may have occurred, but will merely detect the presence of infection in a herd.

My thanks are due to Professor J. W. Emslie for permission to publish the results of these tests, which were carried out in the Veterinary Pathology Department of the Glasgow Veterinary College.

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THE VALUE OF D.D.T. and "666" AS ANTI-KED SHEEP DIPS

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Introduction

THE sheep ked, *Melophagus ovinus, L.*, is of minor veterinary importance since, as far as is known, it transmits no disease. It is the vector of *Trypanosoma melophagium*, but this appears to be non-pathogenic to sheep. To the flockmaster, however, it is of considerable economic importance because staining of the fleece by ked fæces may lower considerably the value of a wool clip. The usual commercial arsenical or phenolic dips appear to be of little value in eradicating keds as, although they are in many cases lethal to adult keds, the fleece does not remain toxic for a length of time sufficient to kill all pupæ which hatch after dipping.

Six ounces of Derris containing 5 per cent. Rotenone in each 100 gallons of water was shown by Cobbett and Smith (1945) to be promising as a ked dip.

Heath (1945) showed that sheep which had been dipped in D.D.T. at a bath concentration of 0.5 per cent. remained free from keds for at least 36 days after dipping. The present investigation was concerned with lower bath concentrations and the possibility of longer protection. Also, the value of "666" (hexachlorcyclohexane) as a ked dip was investigated. While the work was in progress, Rude and Parish (1945) described a large-scale field trial of D.D.T. at a bath concentration of 0.2 per cent., in which 3,417 sheep and 1,558 goats remained free from keds for 90 days after dipping.

Material and Methods

Three groups, each of two crossbred sheep, were dipped in baths of D.D.T. at concentrations of 0.5 per cent., 0.25 per cent. and 0.125 per cent. respectively.

After dipping, all the sheep were placed in a small paddock along with a control group of two undipped sheep. Examination before dipping showed a light infestation of keds on all sheep. Three similar groups of sheep were dipped in "666" at concentrations of 0.5 per cent., 0.25 per cent. and 0.125 per cent., and, along with two control sheep, were run into a paddock close to, and similar to, that carrying the D.D.T.-dipped sheep.

The dips used were prepared from stock emulsions supplied by Dr. J. G. Mitchell, Chemical Research Laboratory, Department of Scientific and Industrial Research. They mixed easily and wetted the fleece well.

On 14 occasions during the 74 days immediately following dipping, a total of 686 live keds caught by hand from sheep running elsewhere were distributed in the fleece of all groups of the D.D.T.-dipped sheep. Forty-three days after dipping, one heavily ked-infested sheep was put into the D.D.T. paddock for 24 hours and, one week later, 12 sheep heavily infested with keds were run along with the dipped sheep for three days. The two control sheep were removed from the experiment on the 74th day, the 0.125 per cent. D.D.T. on the 85th day, and the 0.25 per cent. D.D.T. on the 90th day after dipping. The experiment was discontinued 102 days after dipping.

Because of technical difficulties, dipping with "666" was carried out 44 days later than the D.D.T. dipping, and the period of observation after dipping was 29 days shorter than that of the D.D.T. group. Twelve live keds were put into the fleece of each sheep 30 and 62 days after dipping. Twelve heavily ked-infested sheep were run with the dipped sheep for three days, beginning 40 days after dipping.

As an arbitrary method of estimating the effectiveness of the dips, each sheep was examined for about two minutes at almost daily intervals. All keds found were recorded as "Alive" or "Dead" and all dead keds were removed to obviate the possibility of counting the same ked twice. In every case the fleece was parted longitudinally along the mid-line of the back, and again at a distance of about 6 in. laterally on either side.

Results

TABLE 1.

D.D.T. PADDOCK. DIPPED 3.12.45.

Group	No. of exams.	Period covered by exams.	No. of keds counted ALIVE	DEAD	Average No. of keds counted per examination ALIVE	DEAD
0.5% D.D.T. ...	74	102 days	25 ...	210	0.3 ...	2.8
0.25% D.D.T. ...	63	89 „	19 ...	206	0.3 ...	3.3
0.125% D.D.T. ..	59	84 „	44 ...	192	0.8 ...	3.3
Undipped ...	54	74 „	247 ...	131	416 ...	214

TABLE 2.

"666" PADDOCK. DIPPED 16.1.46.

Group	No. of exams.	Period covered by exams.	No. of keds counted ALIVE	DEAD	Average No. of keds counted per examination ALIVE	DEAD
0.5% "666" ...	57	73 days	10 ...	103	0.2 ...	1.8
0.25% "666" ...	57	73 „	37 ...	81	0.6 ...	1.4
0.125% "666" ..	28	40 „	1 ...	56	0.0 ...	2.0
Undipped ...	45	59 „	535 ...	10	12.0 ...	0.2

Tables 1 and 2 summarise the results obtained from examinations of the D.D.T. and "666" dipped groups respectively. Salient points which are not shown in the tables are :—

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(1) Fifty-four days after dipping, after three days' contact with 12 heavily infested sheep, no live keds could be found on any of the D.D.T.-dipped sheep within three days of removal of the undipped sheep.

(2) The two sheep dipped in 0.5 per cent. D.D.T. were infested with 24 live keds 99 days after dipping. On the following three days, counts were: 5 alive, 15 dead; 1 alive, 2 dead; 1 alive, 0 dead. No live keds had been found on the 0.5 per cent. D.D.T. sheep after control sheep had been removed 74 days after dipping, until this artificially induced infestation 25 days later.

(3) Forty-three days after dipping, after three days' contact with 12 heavily ked-infested sheep, no live keds could be found on any of the "666"-dipped sheep within two days of the removal of the undipped sheep.

(4) The two groups dipped in 0.5 per cent. and 0.25 per cent. "666" were infested with a total of 48 live keds, 62 days after dipping. On the following four days, counts were: 9 alive, 30 dead; 6 alive, 2 dead; 3 alive, 2 dead; 0 alive, 2 dead. No live keds were found on the 0.5 per cent. "666" sheep between two days after this artificial infestation and cessation of examinations, 11 days later.

Discussion

Exact interpretation of the results described above is difficult because transference of keds from undipped control sheep to dipped sheep is probably an almost continuous process, which is accelerated during the collection and penning of sheep prior to examination. Since keds may survive many hours after contact with D.D.T., it is probable that a large proportion of the keds classified as "alive" on the dipped sheep had, in fact, but recently left undipped sheep. This view was borne out by the disappearance of live keds on dipped sheep after undipped sheep had been removed from the experiment. A further complicating factor is that pupæ are unlikely to be affected by the dips. Thus, it is conceivable that a female ked about to deliver a larva (which quickly hardens off into a pupa) may transfer from an undipped to a dipped sheep, "lay" the larva and then die, leaving the pupa to hatch several weeks later.

Bearing in mind these limitations attached to interpretation of results, it seems probable that a bath concentration of 0.125 per cent. D.D.T. or "666" renders sheep fleece toxic to keds for a length of time sufficient to eradicate keds from a flock by means of a single dipping (Heath, 1945). Also, dipping three times per year in a bath concentration of 0.5 per cent. D.D.T. or "666" keeps the fleece perpetually toxic to keds.

Since the ked cannot live off the host animal for more than about 10 days (Cameron, 1940), it appears that a single dipping of an isolated flock in either D.D.T. or "666" at concentrations as low as 0.125 per cent. would be adequate for ked eradication.

Summary

D.D.T. and "666" at bath concentrations of 0.5 per cent., 0.25 per cent. and 0.125 per cent. were used as anti-ked sheep dips, using groups of two sheep each.

A bath concentration of 0.125 per cent. of either substance appears to be sufficient to render fleece toxic over a period of time sufficient to eradicate keds from any isolated group of sheep.

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Acknowledgements

To Dr. H. H. Green, under whose direction this work was carried out.

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SEBORRHŒA OLEOSA EQUINUS

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Wonersh.

Ætiology

For generations this form of dermatitis in horses has been a constant source of worry and loss, more especially in the larger commercial studs employing horses of the heavy vanner and dray types.

It has been held that some horses, particularly Shire cross-breds, have a predisposition to "grease"; that they have a peculiar constitution which tends to allow the pathological process to arise—a so-called "seborrhœic diathesis"—in which the subject is assumed to have some deficiency of enzymes necessary for the proper metabolism of the fatty food materials brought to the sebaceous glands. More recently it has been the vogue to ascribe these conditions to a cutaneous expression of the B-avitaminosis syndrome.

Similarly, arguments have been advanced that the trouble is due to some lack of equilibrium between the various endocrinous glands, and that this gives rise to an excessive secretion.

It is certainly true that human males at about the time of puberty are prone to develop lesions of acne vulgaris; this latter is but a seborrhœa in which the sebum is trapped and cannot obtain exit from the sebaceous ducts. Although it appears to be true that the thicker-skinned, extra coarse-haired type of horse is more prone to the disease, and that horses with little "feather" rarely suffer, and the thin-skinned lighter types and breeds never, it cannot be due to a B-avitaminosis, because affected horses turned out on to a good cattle-fattening pasture for the whole of the grazing season do not improve.

It appears more probable that the coarser skin of half-breds, that are commonly affected, has a more sluggish circulation, that the pores of the sebaceous glands are more papulous and flaccid, and that the skin has not the "tone" of the finer-skinned thoroughbred or lighter types. Moreover, the excess of coarse hair not only harbours and masks any accumulation of dirt, but provides an effective cover from atmospheric influences: an excess of sebum, plus dirt, plus a comparatively even temperature making an excellent nidus for micro-organisms to flourish.

Recent studies in humans suggest that the bottle-bacillus of Unna, variously named as the spores of Mallasez or pityrosporum ovale—a fungus-like organism

which is held to be normally a saprophyte—can become pathogenic in a medium such as the oil secreted from the sebaceous glands, and it then invades the epidermis, to produce a seborrhœic dermatitis.

The fact that sulphanilamide rapidly clears up the condition seems to support the view that some organism which is non-resistant to that drug is responsible for the initiation and exacerbation of the process. For evidence points very strongly to the view, indeed it is now generally accepted, that the bacteriostatic action of the sulphonamide series is due to some interference with the chain of enzymatic processes concerned with bacterial growth. It was found that an extract from yeast was particularly potent in antagonising the bacteriostatic action of the sulphonamides. This extract closely resembled *p*-amino-benzoic acid. The theory is that there is a competition for the enzyme between *p*-amino-benzoic acid and the stereo-chemically similar sulphonamides. If the latter wins the first round with a "knock-out" dose, its ascendancy is maintained. If, however, the growth of the organism has not been completely inhibited from the first "gong," it appears to acquire an enhanced power of synthesising some sulphonamide antagonist such as (amongst others) *p*-amino-benzoic acid, and becoming completely resistant to the drug; a method of protection that trypanosomes can acquire under arsenical dosage.

Many medicaments have been used and advocated as palliatives of the disease, ranging from formalin, through the metallic sulphates and chlorides, down to the urea of urine. Most of these succeed in temporarily inhibiting the discharge for a short time, but all of them suffer from the disability that if they have sufficiently astringent properties to suppress the exudate they invariably leave an indolent ulcerative surface, which quickly reverts to an actual active seborrhœic discharge. I would even hazard the opinion that it is probable that these irritant dressings are finally responsible for the concluding phase of "grapes." It has been said that there are pasture lands upon which affected horses will recover; such ground has mud-poached watering places, and their legs are continually plastered with a sheath of air-excluding clay. It may be that the mud is of the kaolin type and is slightly astringent, or maybe it is merely the exclusion of air from the skin surface, inhibiting the growth of some possibly ærobic micro-organisms which it is suggested are the cause of the disease, that produces a temporary cure.

It would seem to be a logical deduction that since the topical application of sulphanilamide does produce a cure which does not relapse, the casual factor is some undetermined micro-organism.

(1) *The initial lesion* appears to be a localised aggregation of epithelial scales, somewhat reminiscent of glistening bran, which give the impression of being attached at one border to the skin, and are closely packed obliquely to the skin surface.

This area, which commonly is located on either that part of the heel immediately overlaying the lateral cartilage of the os pedis, or on the lateral surface of the sesamoid area of the fetlock, is, at this stage, dry, although the scales when palpated feel somewhat "soapy." The folds of skin behind the pastern and the horizontal wrinkle of skin which is formed when the foot is fully flexed, just above the back of the fetlock, are also sites of election. Occasionally the lateral

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surface of the flexor tendons is the only site, but generally the evolvement of these areas is due to the spreading dermatitis of an unchecked initial lesion. The extensor surface of the legs or foot is hardly ever implicated. The few cases that have been observed seem to arise from an area that has had a small abrasion of the skin, such as is caused by a "tread" when in pair-horse harness, or from a "brushing" injury.

(2) *The second stage* is the exudation of a golden-orange, oily discharge, which issues from an area of skin about the size of a "shilling piece." This continuous discharge is gummy in texture and tends to dry to a darker brownish colour, matting and scabbing the hairs of the leg, the so-called "feather," some little distance from the skin surface. At this early stage the discharge, although "mousey" in odour, has not developed the extraordinarily offensive nature that it acquires later.

In some rarer cases an intermediate stage is encountered, in which the droplets of exuded sebaceous material become congealed and hang from the skin like a curtain of elongated, amber-coloured beads, each about the size of a seed of rye-grass. This condition may not be observed, for it is masked by the feather of the leg, which is not matted or discoloured. Close examination will reveal more or less of an epithelial desquamation consisting of an extrusion from the sebaceous glands and their ducts of horny cells of the epidermis which have not undergone their normal complete fatty degeneration; these show as bran-like scales and crusted, scab-like areas, together with a surrounding active skin inflammation which is discernible.

(3) If untreated, the whole picture of a spreading dermatitis quickly unfolds, the exudation spreads, exfoliation of epithelium appears in the heel and back of the pastern, and the back and sides of the fetlock, even halfway to the point of the hock and flexure of the knee, show evidence, sooner or later, of the various lesions, scaliness, exudation, scabbing, fissures of the skin, usually marked at the back of the pastern and in horizontal sulci above the sesamoids.

The whole skin of the leg from below the knees and hocks in the course of months becomes thickened and indurated. The limb itself is swollen with a lymphatic congestion.

At this stage an untreated, uncleansed case is revolting, with a penetrating odour which is apparent on entry into the stable housing the affected animal.

(4) The fissures in the skin soon ulcerate and granulation tissue is proliferated. This, with the exacerbation and remission of the inflammatory process, acquires a thin pellicle of epithelial tissue and assumes papillomatous, wart-like excrescences, which are termed "grapes."

With the old treatments of astringents of varying strengths and types, the "weeping" areas were checked after the course of months, but always one could observe an ulcerated area demarcated by a white line of epithelial growth, overlooking a shallow crater, which was more or less filled with so-called grapes, varying in size from seeds to actual fruit-like grapes.

The crater contained a greyish, greasy, smegma-like exudate, and had an underlying blueish pigmentation of the skin. In it could be seen the papulous orifices of the seborrhœic glands, many of them so flaccid that their openings are

as large as big pin-heads. Around the crater-edge the skin was rosaceous.

With treatment the first-stage scales are removed and a normal, healthy skin is restored in a few days. The second stage of exudation is also quickly controlled, and very few dressings serve to check the discharge. A week later no traces, except, perhaps, for a mild roseola surrounding a very thin, dry, yellowish, small, adherent scab, mark the site. This scab soon flakes off, and a small, shiny scar, which disappears later, is left.

The third stage responds more readily than one could reasonably expect. Within a week the affected areas are clear of discharge, and although the papulous orifices of the sebaceous glands, inset in a rosy-bordered, blueish-coloured, elephant-like skin, are apparent, it is clear that a reversion to a healthy condition is under way.

When grapes have been present, their epithelial covering tends to acquire a whiter, deeper texture, the whole area slowly loses its macerated pathological appearance, and the fissure slowly heals.

It is only when the gamut of skin changes have terminated, probably with the added impetus of locally applied astringent irritations, in epitheliomata or hypertrophied skin papillæ of more than rice-grain size, that complete healing cannot be expected. Even in aged horses, with the disease of many years' standing, the gradual remission of the chronic dermatological changes is remarkable, and although the formation of fibrous tissue prohibits a return to absolute normality, these animals are no longer eyesores, and become useful working horses, no longer needing the constant daily dressing that was formerly necessary, and without which regularity of attention they were an offence to sight and smell.

The technique of treatment is very simple, and as follows :—

(1) Clip the hair as closely as possible over all areas that show any departure from normal.

(2) Once daily, with soft soap and warm water, cleanse thoroughly, making certain that no discharge, crusts or scabs remain. Rinse well and dry with a clean cloth. Allow to dry out completely in the open air.

(3) Twice daily rub in briskly sulphanilamide powder; some of the first application will become incorporated with a remaining film of discharge and will crumble off; repeat the application until an evenly-dusted appearance of the affected areas is evident.

(4) In a few days, when the area becomes dry, cease the daily washing, but repeat the brisk dressings twice daily until it is certain that no lesion, even the tiniest crust or scale, remains. This period will vary in inverse proportion to the age of the lesions, from a few days to some weeks.

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SOME NOTES TAKEN DURING THE SESSIONS OF THE UNITED STATES LIVESTOCK SANITARY ASSOCIATION, HELD IN CHICAGO, DEC., 5-7, 1945

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DR. W. H. BOYNTON, in his "Report of Advisory Committee on Anaplasmosis," brought out the following points:

(1) A combination of D.D.T. and rotenone seems to be best as an application to cattle to assist in the prevention of the spread of this disease.

(2) Cobalt chloride, sulphathiazole, and neosalvarsan seem to give the best results in hastening the recovery of acute cases. Cobalt chloride is said to increase or stimulate red blood cell formation in the bone marrow.

(3) In view of the fact that recovered cases are "carriers," it was suggested that these animals be branded.

In the discussion, Schmidt, of Texas, did not agree that blood transfusions are always indicated in the handling of anaplasmosis. Only if there is anoxia is a blood transfusion desirable. The blood used for transfusion might possibly have been obtained from a "carrier," because these latter are clinically unrecognisable, and thus a mass infection would be imposed on top of the existing infection.

K. C. Seegar, Assistant Professor, University of Delaware, in discussing "The Response of Induced and Natural Eimeria Tenella Infections to Sulphaguanidine," said that he had a 2.7 per cent. death loss in sulphaguanidine treated birds, and 80 per cent. loss in the controls. They used 1 per cent. sulphaguanidine in the feed for five days.

In this research project the birds were artificially inoculated. These birds were found to be immune afterwards. On the other hand, birds exposed to natural infection must be treated repeatedly with the sulphaguanidine, because these birds were in various stages of infection at the time of treatment. For these latter birds they gave 0.75 per cent. of sulphaguanidine in the feed for a few days, and repeated at eight-day intervals. This gives a high degree of protection, and the birds thus treated had increased weights over those not treated.

Dr. James M. Murphy, New Jersey Experiment Station, reported on "The Incidence of Streptococcal and Staphylococcal Bovine Udder Infections in Relation to Predisposing Factors." He stated that as animals grow older there is a tendency for streptococcic infections in the udder to increase, while staphylococcic infections usually remain on a constant level.

Injury seems to be a factor in increasing streptococcic infections, but it is not a major factor.

Increasing size of the teat canal as the animal grows older is believed to favour infection, but this theory has not been substantiated.

Hormones were not found to be factors in increased streptococcal infection.

The most important factor in the production of udder infections was found to be contact with mass infections.

He indicated that there is a feeling that non-hemolytic staphylococci may be

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normal inhabitants of the udder. The dangerous staphylococci that are destructive to erythrocytes are usually the most pathogenic.

Streptococci undoubtedly enter through the teat canal, and evidence is accumulating that staphylococci may also enter by this route.

Injury to the end of the teat might increase the danger of streptococcal infection, but such an injury is not a major necessity. This is also true regarding teat patency.

The author believes that the first few strips of milk should be used for making an examination, instead of discarding these strippings. He stated that the first strippings contained no more pathogenic organisms, though probably a greater variety of organisms, than later strippings.

It has been demonstrated that some udders have a natural inhibitory action on the growth of organisms.

Dr. F. R. Beaudette, New Jersey Agricultural Experiment Station, reported on "Newcastle Disease in New Jersey." He stated that this is a virus disease, and that it is easiest to isolate the virus during the early stages of the disease. The liver, brain, spleen and blood serum were used for inoculation purposes. The inoculation material is frozen, and kept so until it is to be used for inoculation. He uses a small amount of inoculum in 1 c.c. of broth. Of this amount 1/10 c.c. is injected into 10 to 12-day-old chick embryos. The liver of the embryo, and sometimes allantoic material, is used for future inoculation purposes. If the embryo did not die it was considered as not infected, though its liver was saved for inoculation purposes. Some of the suspects were carried to the seventh generation before becoming declared free of the virus. About one-third of the brain samples were found to be positive, and one-half of the spleen tissues were positive on the first test. Some of the others became positive on later tests. Of the 26 tissues, 19 were positive or they killed every embryo on the first test. Sometimes both brain and spleen from the same embryo are positive, but the brain may be positive and spleen from the same chick negative, or the reverse.

In New Jersey the disease has been identified in 7 out of the 21 counties of the state—it is in southern, middle and north New Jersey. The disease was first diagnosed by testing it against a known antiserum.

It was recommended to slaughter all birds, turkeys, guinea-hens, ducks, and geese, on infected farms. It was stated that adult birds shipped interstate and the return of egg cases and crates are modes of spread of the disease. The outstanding symptom of the disease is that of a respiratory condition.

Dr. Benjamin Schwartz, Bureau of Animal Industry, reported on "Some War-time Developments in Controlling Parasites of Livestock." He stated that the standard wash in grub control still consists of 12 ounces of derris or cube having 5 per cent. rotenone, and 4 ounces of laundry soap and 1 gallon of water.

Grub dusts no longer have sulphur in them. They consist of one part of derris and two parts of some fine inert powder.

For spraying purposes he recommended 7½ pounds of derris in 100 gallons of water. Nothing else is used in this spray.

In order to control both grubs and lice with a dip, he recommended 10 pounds of derris, 2 ounces of a sodium salt, and 100 gallons of water.

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A second dipping is necessary, but only one part of derris need be used, because lice nits are much more susceptible to rotenone than are grubs.

Sheep ticks may be controlled by rotenone.

Schwartz considers the phenothiazine-salt mixture for the control of gastro-intestinal parasites in sheep as of second importance only to the discovery of phenothiazine itself. He said that one to nine phenothiazine in salt is both curative and prophylactic. He recommended first to give adult sheep 1 ounce of phenothiazine, and lambs weighing less than 60 pounds ½ ounce. Give these full doses in the spring and then the phenothiazine-salt mixture for the remainder of the summer. The breeding flock should have another full dose at the beginning of winter.

As an agent for the removal of flukes he recommended a mixture consisting of 1 pound hexachlorethane, 1½ ounces bentonite, ¼ ounce flour, and 25 ounces of water (the flour is added to keep the other agents in suspension). 6½-ounce doses of this for cattle and one-half of this dosage for calves will remove many liver flukes, provided the liver is not already too badly damaged.

He stated that phenothiazine is as toxic in the treatment of ascariasis in swine as is oil of chenopodium, and that it is not as effective as oil of chenopodium.

He also stated that one part of sodium fluoride by weight in 90 parts of feed will remove from 90 to 100 per cent. ascarides in swine. Sodium fluoride is not particularly toxic for swine if used in the dosage given for one or two days. Sodium fluoride is the agent of choice for the removal of ascarides in swine.

He also stated that whey or powdered skim milk fed intermittently seemed to be an important article of diet in preventing swine parasites.

The "Report of the Committee on Parasitic Diseases," by Dr. Russell E. Rebrassier, of Ohio State University, brought out some of the following points:

D.D.T. is the most valuable parasiticide developed during recent months.

Dusting powders containing 4 per cent. D.D.T. are effective against lice, fleas, etc., or a 1 per cent. wash is equally effective. In these dosages there is no toxicity.

The report indicated that there is ground for the belief that D.D.T. may be effective against all mange mites.

The report recommended 1 to 7 phenothiazine-salt mixture for parasitism in sheep.

Then the report stated that sodium fluoride 1 per cent. of the daily feed by weight will remove 90 per cent. of swine ascarides.

Sodium borate administered for 4 or 5 days, and beginning as late as 4 days after exposure, seems to be of value in the handling of poultry coccidiosis.

Dr. R. Fenstermacher, of the University of Minnesota, reported on "Pitch Poisoning in Swine." He indicated that this condition is especially common in communities where gun clubs are stationed and where clay pigeons are used. Degeneration of the liver is the commonest lesion. Experimentally, 50 grams per day of clay pigeon produced death in about 10 days with gross liver lesions. The addition of 25 grams of lead shot for 5 days did not change the clinical or post-mortem picture. Coal tar pitch used in the sealing of a gas line has also been incriminated. Frequently there are no clinical symptoms until death is

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impending, and then there occasionally is icterus, weakness, and anorexia. On post-mortem examination pigs dead of this disease are found to have a greatly enlarged and engorged liver with a peculiar "speckled" appearance. Pigs under experimental conditions, when finally killed, may show only marginal liver changes. In one investigated outbreak it was 35 years ago since a gun club had operated in a pasture in which the pigs became affected with pitch poisoning.

Dr. C. C. Morrill, of the University of Illinois, reported on "Swine Erysipelas." He stated that the order of frequency of evidences of erysipelas infection observed by him were as follows: Kidneys 83 per cent., spleen 75 per cent., liver 66 per cent., and joints 35 per cent.

Dr. Kernkamp, of the University of Minnesota, presented the "Report of the Committee on Transmissible Diseases of Swine." The report indicated that in 1945 the prevalence of swine diseases in the corn belt area was decreased as compared with 1944. In all states excepting Illinois swine erysipelas was on the decrease. In all states of the corn belt area brucellosis was on the increase. Five of the corn belt states had more tuberculosis in 1945 than in 1944. The enteritis complex had been in a static condition, and this was also true in regard to parasitic conditions.

Dr. Harold M. Johnson, Director, Rabies Laboratory, Rockefeller Foundation, Montgomery, Alabama, reported on "Experimental and Field Studies of Canine Rabies Vaccination." When the single injection method of vaccination was used the vaccinated dogs were significantly protected as compared with controls. Twenty per cent. ovine vaccine was used.

In the three injection method the vaccinated had 100 per cent. protection, though the loss amongst controls was very high. Here, also, a vaccine containing 20 per cent. ovine brain tissue in a phenolised menstruum was used.

In Alabama there was much less human rabies when dog vaccination was enforced. Non-vaccinated dogs should be impounded and killed and all others should be vaccinated if rabies is to be controlled. Non-vaccinated dogs should be kept under absolute control for at least three months following the appearance of the last case of rabies.

Dr. H. W. Schoening, of the Bureau of Animal Industry, presented the "Report of the Committee on Rabies." This report indicated that 10,540 cases of rabies were reported in 1942 to the Bureau of Animal Industry.

The present-day rabies vaccine is a very much improved product as compared with that of former years. The Habel test is used to determine the efficacy of the vaccine. The report recommended that 30 days after vaccination the vaccinated dogs be released from all quarantine restrictions. During these 30 days all strays and uncontrolled non-vaccinated dogs should be picked up. The non-vaccinated dogs are to be kept under absolute control for 90 days following the appearance in that community of the last case of rabies. Results of field and laboratory tests warrant the recommendation that the one injection method of vaccination be used as an adjunct in rabies control methods.

Dr. A. E. Wight, of the Bureau of Animal Industry, presented the "Progress and Status of Co-operative Tuberculosis Eradication Among Livestock." He stated that one Eastern county for the first time in area testing was removed from the modified accredited area because of increased tuberculosis there.

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He also stated that it had been a policy when tuberculosis in a county falls to lower than .2 per cent. to continue that county on the modified accredited list without further testing for at least six years.

The "no-lesion" reactors continued to be a source of trouble, but for the fiscal year 1944-45 it was lowest amongst reactors since 1938.

Dr. Alexander Zeissig, of Cornell University, reported on "A Study of the Result of Retesting a Group of Tuberculin Test Reactors." He stated:

(1) Reactors will give a good second reaction.

(2) Reactors, because of skin lesions, do not react nearly so well on a retest. Only 42 per cent.

(3) The "no-visible" lesion reactors seldom react to a second test.

He brought out that a tuberculous animal usually remains sensitised for a long time.

The allergic state is not nearly so prolonged in the healed or calcified patient.

In problem herds inject subcutaneously 4 c.c. of intradermal tuberculin. Take at least 2 pre-injection temperatures, and commence to take post-injection temperatures commencing 2 hours after the injection, and continue hourly for at least 18 hours.

Dr. Howard W. Johnson, United States Bureau of Animal Industry, Auburn, Alabama, reported on "The Johne's Disease Problem and the Relationship of Johne's Disease to the Nonvisible Lesion Tuberculin Test Reactor."

He stated that it has been determined in Russia that the Johne's organism remains viable in water and soil for at least 11 months. In England it was found viable in water after 13 months, in manure it has been found viable after 246 days. He classified herds as follows:

(1) Herds in which 20 per cent. have Johne's disease, and in which this is manifested clinically by diarrhœa and emaciation.

(2) Herds in which only an occasional animal shows symptoms.

(3) Herds in which animals are positive to Johnin, but which show no lesions on post-mortem examination and in which no clinical symptoms are exhibited.

He declared that Johnin in many cases in the past has not been very potent, and therefore a constant effort should be made to improve this product.

He also stated that out of a little more than eight million cattle tested for T.B. in 1944-45, over one-half million showed no lesions on post-mortem. He expressed it as his belief that infection either with the avian organism or with the Johne's disease organism are most frequently responsible for the no-lesion T.B. reactor.

It was also suggested that tuberculin and Johnin might each have foreign allergens that will occasionally cause reactions for either disease. He suggested that research workers should try to eliminate these non-specific allergens.

Dr. William H. Feldman, of the Mayo Foundation, had prepared the "Report of the Committee on Tuberculosis," but as he was absent because of an attack of influenza the report was read by Dr. W. A. Hagan.

The report indicated that in some Southern states only small parts of herds had been retested, with the result that some cattle have not been retested for 12 years.

In regard to no-lesion reactors, the report indicated that "it is doubted that

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tuberculin is not always absolutely specific, but no evidence can be cited that is considered to be authoritative." It has even been suggested that reagents prepared from other than the tubercle organism may at times incite a reaction similar to tuberculin.

The report stated that the bovine tuberculosis organism can cause any kind of human tuberculosis, and that at least 10 per cent. of all human tuberculosis is of bovine origin. It was mentioned that several hundred cases of pulmonary tuberculosis in humans had been demonstrated to be of the bovine type. The report admitted that the human digestive tract is the principal portal of entry of the bovine organism.

The report also recommended that because the avian organism is such a complicating factor it should be more intensively studied.

Lt.-Col. R. W. Rushmore reported on "Communicable Animal Disease Problems Encountered in Italy." He stated that amongst others, the following diseases were noticed in Italy: Foot and mouth disease, anthrax, tuberculosis, swine-pox, Brucellosis, glanders, hog cholera, rabies, dourine, Newcastle disease, and others.

For foot and mouth disease in Italy vaccination is practiced. It immunises the animal for six months. It consisted in harvesting the virus from the lesions found at the height of the disease and attenuating this with a solution of aluminium hydroxide. The animal to be immunised was inoculated with this material.

In Italy Brucella melitensis was found in sheep as well as in goats.

In Italy they used a non-virulent erysipelas vaccine with what appeared to be good results. This vaccine was valueless in exposed or infected herds.

The dourine in Italy came from captured animals—17 stallions were found to be affected.

Dr. William C. Reeves, of the William Hooper Foundation for Medical Research, reported on "Observations on the Natural History of Western Equine Encephalomyelitis." He stated that the Culex tarsales and the domesticated fowl are important factors in the distribution of western equine encephalomyelitis virus.

Eighty thousand chicken mites examined and obtained from flocks in which equine encephalomyelitis was present failed to disclose the virus in the mites.

Hibernating mosquitoes did not have virus.

One conclusion was that the true reservoir of sleeping sickness virus has not yet been found. He mentioned the triatoma of Kansas State College, but considered it of secondary importance. He stated that until the true reservoir is found efforts to control equine encephalomyelitis are not likely to prove effective.

D.D.T. sprayed on inside walls of barns, chicken coops, chicken houses, etc., may reduce the number of vectors, because the residue of D.D.T. in buildings will control mosquitoes for several months.

Shahan, of the United States Bureau of Animal Industry, in discussing Reeve's paper, said that it has not been possible to recover the equine encephalomyelitis virus from inoculated cattle or sheep.

Schroeder, of Pearl River, New York, presented the "Report of the Committee on Miscellaneous Transmissible Diseases." This committee recommended

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the establishment of Bureau of Animal Industry diagnostic laboratories in various parts of the United States. These laboratories were also to serve as Bureaus of Vital Statistics.

The committee also recommended the establishment by the United States Livestock Sanitary Association of a committee on Vital Statistics.

A. B. Crawford, of the Bureau of Animal Industry, reported on "The Present Status of Bureau Control of Brucella Vaccine and Antigen Production." He stated that the Bureau of Animal Industry supplies all commercial firms with smooth cultures every two months.

The No. 19 vaccine contains five billion organisms per c.c. and is marketed in one dose bottles.

The expiration date is now three months.

Dried vaccine is prepared from frozen vaccine and under a high vacuum. This dried vaccine has an expiration date of one year. It is still in the experimental stage.

Brucella abortus stained antigen is patented by the Bureau of Animal Industry and because of this the Bureau exercises control over it, but under law the Bureau has no control over any other antigen.

Winters, Assistant Director of the Bureau of Animal Industry in New York, reported on "Data Related to Extent of Post Vaccination Agglutination Reaction in Calves." "The New York Plan to Control Brucellosis." He stated that 3½ per cent. of their vaccinates still showed positive reaction at two years of age and 1 per cent. were still positive at the second freshening.

He believes that the vaccination gives greatest protection when applied at six to eight months of age.

New York recommends adult vaccination in badly infected herds.

Dr. A. E. Wight reported on Brucellosis for the Bureau of Animal Industry. This report was an "extra," because Mr. Core, President, National Ayrshire Breeders' Association, failed to appear with his paper entitled "The Control and Eradication of Brucellosis from a Dairyman's Point of View."

Doctor Wight stated that of the tests reported to the Bureau, the percentage was 4.7, compared with 4.3 for the preceding year. He said that 524 counties are now in the modified accredited area. During the year 85 counties were removed from the accredited group.

He stated that in the official programme during the past year 56,000 additional cattle in 26 states had been vaccinated and as a whole the results had been encouraging.

Dr. Carl F. Jordan, of the Iowa State Board of Health, reported on "The Control and Eradication of Brucellosis in Animals from the Standpoint of Human Health."

In Iowa male farm workers show a morbidity six times higher than female farm workers.

Out of 51 strains examined between October 1, 1945, and December 1, 1945, 23 were porcine, 13 abortus, and 15 melitensis.

He stated that Forbes of Johns Hopkins recognises three types of Brucellosis in man as follows: The septicemic form, the local form, and the lymphogranulomatous. The latter is the chronic type in man.

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Dr. Glenn C. Holm, State Veterinarian of Idaho, reported on "Experimental Work on Brucella Abortus Vaccination of Swine." One thousand, one hundred and eighty-one animals were studied during a five-year period.

Out of 85 original reactors, three boars and two sows became negative. Clean gilts bred to these boars did not contract the infection.

Young animals do not remain positive reactors for as long as those swine that contracted the infection later in life.

The pigs were vaccinated with 6 mils. of Brucella abortus vaccine. The pigs were from 51 to 63 days of age. These animals never did develop a high blood titre and a few developed no titre at all. In older animals the blood titre was 1 to 80 and was very persistent.

These vaccinated animals were challenged with oral, conjunctival and vaginal cultures; the conjunctival reaction was the most severe and, therefore, this method of challenging will be used from now on.

Smith, State Veterinarian of New Hampshire, presented the "Report of the Committee on Brucellosis." Amongst other things, the report indicated that the vaccination of pregnant animals is more frequently followed by abortion as the stage of pregnancy is advanced.

The report recommended that Brucellosis in swine should be carefully checked as it is accumulating evidence that swine Brucellosis is an important factor in the production of cattle Brucellosis.

Officers elected for the ensuing year are as follows: Dr. William Moore, State Veterinarian of North Carolina, President; Mr. Will J. Miller, Kansas Livestock Sanitary Commissioner, First Vice-President; Dr. J. V. Knapp, State Veterinarian of Florida, Second Vice-President; Dr. R. A. Hendershott, State Veterinarian of New Jersey, was continued as Secretary-Treasurer.

TWENTY-SIXTH ANNUAL CONFERENCE OF RESEARCH WORKERS IN ANIMAL DISEASES IN NORTH AMERICA, CHICAGO, ILLINOIS,* DECEMBER, 4, 1945

GASSNER, of Ft. Collins, reported on "The Effects of Feeding Cow Fæces, Methyl Testosterone and androstenedione on the Combs and Gonads of White Leghorn Chicks." He stated that adding cow fæces to the extent of 10 per cent. to an otherwise adequate diet for poultry causes comb growth, but at the same time there was a loss of interstitial testicular tissue, and that this may be a cause of future breeding troubles.

He also stated that if a riboflavin deficiency exists in the diet of poultry that cow manure may supply this deficiency.

Burmester, of East Lansing, Michigan, reported on "Manifestations of a Filterable Agent in a Fowl Lymphoid Tumour." He stated that lymphoid muscle or lymphoid liver injected intravenously or intraperitoneally would produce tumours in 7 days. If cell-free extracts are used for inoculation purposes, the formation of lymphoid tumours was delayed for 60 to 180 days.

*By courtesy of Dr. A. H. Quin, Kansas City.

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McNutt, of Iowa, reported on "Some Viruses Isolated From Swine." He stated that human influenza virus Type A produced 100 per cent. abortions in pregnant guinea-pigs. Also that swine flu. virus produced death of chick embryos in 24 hours; that the human strain of influenza virus produced the death of chicken embryos in 48 hours, and an unknown strain in 3 days.

Morrill, of Urbana, Illinois, reported on "Does Fasting Cause Uræmia in Newborn Pigs?" He believes that the condition observed by him was a hypoglycæmia with changes in the liver glycogen and blood sugar, and that the observed increase in uric acid is of no particular significance. Keeping affected pigs warm and giving them glucose was found to be very helpful.

Kernkamp, of Minnesota, reported on "Clinical and Pathological Observations on a 'Toxæmia' in Suckling Pigs." His experiments were conducted at the Hormel Packing Plant, in Austin, Minnesota. During the project they had an average of a litter a day with a mortality as high as 80 per cent. Post-mortem examination disclosed that the supra-renals, the liver, and the kidneys were enlarged and discoloured. No conclusions were reached—the disease was simply diagnosed a toxæmia.

Roepke, of Minnesota, reported on "Analytical Blood Studies on a 'Toxæmia' in Suckling Pigs." This report concerns the same pigs reported on by Kernkamp in the preceding paragraph. Roepke stated that the blood urea nitrogen was greatly increased from the day of birth until death 10 days later. The blood sugar varied and was not considered of diagnostic value. He did not consider that the uræmia and hypoglycæmia are the primary causes of the trouble, but the primary cause is not known. All the pigs were strong and healthy at birth. Kernkamp said that the normal death rate in pigs is 30 per cent. The sows had an adequate ration, including pasture.

Doyle, of Indiana, reported on "A Transmissible Gastro-enteritis in Pigs." He stated that the clinical symptoms consist of vomiting, diarrhœa, and dehydration observed in baby pigs. The symptoms in older pigs were similar, but while the death loss in the baby pigs varied from 90 to 95 per cent., there were no deaths in older affected animals. The incubation period of the disease was as low as 18 hours, with death in about 72 hours. The disease seldom occurs a second time on the same farm. On post-mortem most of the pigs have an inflammation of the stomach and intestine, or occasionally only of the intestines, and in a few cases there was neither gastritis nor enteritis. Small pieces of gastro-intestinal tissues of sick pigs placed in the mouths of healthy pigs is soon followed by the disease in the latter. The disease could also be reproduced by a filtrate. Frozen filtrate stored in a refrigerator was still virulent 80 days later. Hog cholera virus as determined by serum and immunisation tests plays no part in the condition. Pigs 2 or 3 days old died more frequently from this disease than those not affected until after they were 10 or 11 days old.

Cross, of Ft. Collins, reported on "The Use of Sulphur in the Control of Enterotoxæmia in Feed-lot Lambs." Research indicated that sulphur used in treating coccidiosis of lambs caused those lambs to do better than those not receiving sulphur. They used as much as 7.5 grams of sulphur per lamb per day.

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Losses in the sulphur-fed lambs were significantly lower than those not getting sulphur.

Gilman, of New York, reported on "The Occurrence and Survival of *Brucella abortus* in Chedder and in Limburger Cheese." He found that the *Brucella abortus* organisms were viable in cheese at the end of 3 months, and then gradually died out.

In commercial vats Brucella organisms are found in 11 out of 79 samples of milk, but in from 41 to 84 days later there were no viable Brucella organisms.

From the public health standpoint, it may be concluded that 60 days of storage will make all cheese safe from the standpoint of *Brucella abortus.*

Hutchings, of Lafayette, Indiana, reported on "Studies on Brucellosis in Swine: Brucella Infection in the Boar." Using 6 pigs, all Brucella reactors, he recovered *Brucella suis* from the semen from 68 per cent. Some of the boars lost the agglutination reaction, but continued to discharge *Brucella suis* with the semen.

On post-mortem the *Brucella suis* was recovered from only one testicle, and it had shown clinical evidence of orchitis.

Huddleson, of Michigan State, reported on "The Mucoid and Wax-like Growth Phases of Brucella and their Biological Significance." In the mucoid phase the *Brucella abortus* organism is practically non-virulent, though in other organisms having a mucoid stage it is usually the most virulent stage.

The mucoid phase always comes from smooth colonies and never from rough.

LeGrow, of Ithaca, New York, reported on "The Relation of the Opsonic Index to Brucella Infection and Immunity in Cattle." He stated that chronic reactors usually show a high opsonic index, and resistant animals or those having lost the positive reaction frequently have a low opsonic index.

Ross Brown, of East Lansing, Michigan, reported on "Six Months' Results of Daily Mastitis Testing of an Infected and Non-infected Cow." Two cows were used, one mastitis infected and the other mastitis non-infected. In all cases the leucocyte count, the Hotis test reaction, the thybromal test, the chloride test, and cultural tests were higher in the infected animal, but not consistently so.

He stated that there was good ground for the belief that the blood agar plate was the most reliable test.

Merchant and Packer, of Iowa State College, reported on "The Sensitivity of Bovine Mastitis Bacteria to Penicillin." The tests were *in vitro.* Forty-two strains from 80 herds have been studied. Eight organisms have been studied, including pyogenes, dysgalactia, agalactia, uberis, *Brucella abortus,* and some staphylococci. The pyogenes was most susceptible to penicillin. Streptococci were more susceptible than staphylococci, and abortus was more resistant. Of the latter, old cultures were much less resistant than new cultures.

Plastridge, of Storrs, Connecticut, reported on "Influence of Volume of Carrier on the Efficacy of Penicillin in Eliminating *Str. agalactiæ* from the Bovine Udder." They found that mineral oil was more efficient than water for the suspension of the penicillin. The mineral oil suspension gave the best results in the treatment of mastitis.

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Also, as the volume of single infections were increased from 25 mils. to 100 mils. and the Oxford units from 25,000 to 100,000 the results were increasingly better for the handling of agalactia.

Cunkelman, of East Lansing, Michigan, reported on "Penicillin Therapy in Field Studies of Bovine Mastitis Control." He used 42,000 units of penicillin dissolved in 25 c.c. of sterile water. The grain feed was reduced, and the animal was kept stabled for 4 days after the infusion was made. Fifteen of 19 cows so treated became bacteriologically negative after one infusion, two required two infusions, and two required three infusions.

One cow 8 or 9 hours after the infusion showed a severe reaction such as anorexia, pulse 90, temperature 103°, tremors of muscles of the flank and thigh. Ninety-six hours later the cow was normal, though giving only 50 per cent. milk flow. By strip-cup test and microscopic test, the cow at this time was negative. This cow at the time of treatment was receiving mouldy ensilage as a part of its feed, and the thought was expressed that she may have been sensitised.

At 3 to 8-week intervals all treated cows have passed negative tests. The treatment was used at all stages of lactation, and sometimes there was some fibrosis.

Schalm, of Berkeley, California, reported on "Treatment of Bovine Mastitis with Penicillin." He stated that in his opinion if the mastitis organism reappeared in the milk inside of 2 months the treatment was a failure. The best results were obtained from 50,000 units of penicillin left in the udder for 24 hours. The results were 100 per cent. successful if the infection was *Streptococcus agalactia*, and in the same strength there was complete failure if the infection was due to *Staphylococcus aureus*. Schalm recommended adequate doses because repeated small doses may make the animal penicillin resistant.

In the discussion following the presentation of penicillin treatments for mastitis, Dr. Bryan, of the clinical staff of Michigan State College, said that he wanted to add to the confusion by stating that in their clinic they got equally good results as those that had been reported if they used 1,000 Oxford units in 1 c.c. of water.

The final action of the research group was the election of officers, with the following results: Biester, of Ames was elected president; Stubbs, of Pennsylvania, was elected vice-president; Boyd, of Minnesota, was elected a member of the administrative council.

Feldman, the regular secretary-treasurer, was not present, because of an attack of influenza.

R. R. DYKSTRA,
Dean, School of Veterinary Medicine.

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NEW ZEALAND NATIONAL VETERINARY SERVICE

A NATIONAL veterinary service for New Zealand is to be established under legislation to be introduced during the coming session of Parliament. If the current proposals are put into effect a Central Council will be set up with the following representation: Government (2), Dominion Federation of Farmers' Veterinary Services (2), Dairy Board (2), Meat Producers' Board (1), Wool Board (1), Veterinary Association (1).

The function of the Council will be to organise efficient veterinary services for all the livestock owners of the Dominion. It will have complete control of central funds, to be provided by a levy on animal products subsidised by the Government, probably at the rate of £1 for £1.

The Council will encourage the formation of more veterinary clubs, to which it may grant subsidies to ensure their stability. It will endeavour to ensure that sufficient veterinarians are trained, and to that end it may grant bursaries to selected students. It will seek to promote the fullest possible collaboration between all interested organisations with a view to increasing the efficiency of animal husbandry, and it may appoint officers to arrange for extension work in animal health and production.

It is expected that the scheme will broadly follow the recommendations made in the majority report of the Veterinary Services Committee last year. That report suggested the following basis for the levies: 400,000,000 lb. of butterfat at 0.02d. per lb., £33,333; 1,000,000 bales of wool at 1d. a bale, £4,667; 12,000,000 fat lambs at ¼d. a head, £12,500; total, £50,000. A £1 Government subsidy would raise the total to £101,000.

ERRATUM—ARTICLE BY SORENSEN ON "TRICHOMONAS"

On page 74: (4) of the technique was omitted. This should read—"(4) The dried smear is then immersed for one minute in absolute alcohol."

On page 76, second line from the bottom: The end word should be "cytostome," not "cytoplasm."

On page 78, line 23 from the top: The last figure on this line should be 24, and not 25, as the table includes only 24 measured individuals.

Publishers' Notices

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